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Seroprevalence of PCV2 in feeding pigs before, during and after the PMWS epizooty in Switzerland

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Für meine Familie

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Abstract

In Switzerland, the PMWS epizooty started in 2003 and reached the zenith in 2008. After mass vaccination of piglets in the third week of life in 2008/09, PMWS decreased dramatically. PCV2-specific antibody profiles of 200 randomly selected serum samples of slaughter pigs of the preepizootic stage (1996/97) were compared with 200 serum samples collected nearly at the top of the epizooty (2006) and with samples collected in 2011, two years after after mass vaccination. In 1996/97, PCV2 antibody titres were low throughout Switzerland with modest positive samples. In 2006, almost all of the samples were positive with much higher titres. In 2011, antibody titres decreased to a level as low as the preepizootic level. Additionally, 50 samples of each period were analyzed for IgM concentration. Only two samples of the year 2006 contained IgM antibodies.

Keywords: PCV2, Switzerland, vaccination, epizooty, seroprevalence

Zusammenfassung

Die Schweizer PMWS Epizootie begann im Jahre 2003 und erreichte ihren Zenit im 2008. Nach der Einführung der flächendeckenden Impfung der Ferkel in der 3. Lebenwoche in den Jahren 2008/09 reduzierte sich die Anzahl der PMWS – Fälle drastisch. In der vorliegenden Arbeit wurden PCV2-spezifische Antikörpertiter von 200 zufällig ausgewählten Schlachtschweineseren der Präepizootie (1996/97) mit je 200 Serumproben aus den Jahren 2006, fast auf dem Höhepunkt der Epizootie, und 2011, nach der flächendeckenden Impfung, verglichen. In den Jahren 1996/97 hatten wenige Tiere einen positiven PCV2 – Antikörpertiter und die Titer waren tief. In 2006 waren fast alle Proben positiv auf PCV2 – Antikörper und die Antikörpertitrer waren um ein Vielfaches höher als zuvor. Im Jahre 2011 sanken die Titer wieder fast auf das präepizootische Niveau ab.
In einer zweiten Untersuchung wurden je 50 Serumproben aller drei Zeitpunkte auf das Vorhandensein von anti PCV2-IgM Antikörper analysiert. Zwei Proben aus dem Jahr 2006 waren positiv für IgM.

Schlüsselworte: PCV2, Schweiz, Impfung, Epizootie, Seroprävalenz

1 Introduction

Porcine Circovirus Type 2 (PCV2) is one of the most significant pathogen in pig production worldwide. The virus is involved in several syndromes summarized as porcine circovirus type 2 diseases (PCVDs) (Segalés et al., 2005). Postweaning multisystemic wasting syndrome (PMWS), nowadays termed as porcine circovirus type 2–systemic diseases (PCV2-SD), was the most relevant and devastating disease, described first in Canada by Harding and Clark in 1997. Since then, PCV2-SD has spread worldwide, potentially among the trade ways (Segalés et al., 2013). However, PCV2 is the causative agent for PCVDs and the synergy with other factors is extensively discussed from different points of view involving other infectious pathogens (Opriessnig et al., 2007; Segalés et al., 2005), bad husbandry and management (Woodbine et al., 2007) and other stress-factors (Baumgartner et al., 2012) respectively immunosuppression (Klausmann et al., 2015). PCV2 is ubiquitous and can be isolated from both healthy and diseased pigs (Allan and Ellis, 2000). Evenmore, PCV2-DNA could be detected in 100% of the fetuses of cinical healthy sows slaughterd at 80 – 105 gestation day (Sydler et al., 2016).

Thus, PCV2-specific antibodies are ubiquitous and therefore PCV2-serology has no diagnostic value. However, analysis of seroprofiles is useful to follow maternal antibody titres, to fix the time of infection or seroconversion and to measure the efficacy of vaccination.

PCV2-SD affected and healthy animals seroconvert at the same time point or later and PCV2-specific IgGs are slightly lower in PCV2-SD-affected pigs compared to healthy pigs (Meerts et al., 2006). The major immunological differences are a low level of neutralizing antibodies, high production of IL-10 and poor IFN-γ response in sick pigs (Meerts et al., 2006; Meng, 2013). An increased virus replication in affected pigs was observed (Meerts et al., 2005). PCV2-SD affected pigs seem to be unable to recognize or to produce antibodies against the epitopes that are involved in the virus neutralization (Meerts et al., 2006). In asymptomatic pigs, the maternal antibodies decrease from 3rd to 11th week of life and the naturally acquired PCV2-specific antibodies increase until slaughtering (Ramamoorthy and Meng, 2008).

Experimental vaccination studies have shown elevated PCV2-neutralizing antibodies (Fort et al., 2007; Solis Worsfold et al., 2015), significantly shorter and lower viremia in vaccinated than in unvaccinated pigs (Cline et al., 2008; Fort et al., 2008) and a higher proportion of CD4(+) CD8(+) INF-γ(+) lymphocyte subsets in vaccinated piglets (Oh et al., 2014). Viral load reduction and lower percentage of infected pigs are perceived as corner stones in order to control the clinical manifestations (Feng et al., 2014). In subclinically infected herds, slaughter pigs showed higher PCV2-specific antibody titres compared to pigs vaccinated with a subunit vaccine in the third week of life (Fachinger et al., 2008; Kixmöller et al., 2008; Solis Worsfold et al., 2015). The authors explained this finding with shorter and lower viremia levels in vaccinated pigs, avoiding an extensive PCV2-specific antibody production. Vaccination against PCV2 is enormously effective to prevent clinical manifestations, but is not able to avoid PCV2 infection (Fort et al., 2009). PCV2 vaccines are used either in piglets at 3 - 4 weeks of age or in sows in the late stage of gestation to enrich maternal antibodies in the colostrum for passive immunization. Nowadays, subunit piglet vaccines containing the capsid protein of PCV2a are the most frequently used worldwide. Vaccination studies demonstrated that vaccination with subunit vaccines are activating both humoral and cellular immune response (Martelli et al., 2011; Fort et al., 2012), in spite of the fact that all fetal thymi are latently infected with PCV2 (Sydler et al., 2016). The immune system of vaccinated pigs recognizes a large polypeptide fragment of the capsid protein (CP 43-233) and this region has a strong virus neutralizing activity (Trible et al., 2011). This may be the reason why only vaccinated pigs are protected against disease in a challenge model (Trible et al., 2011). In contrast, antibodies of diseased pigs are mainly directed against an immunodominant oligopeptide of the capsid protein (CP 169-180), without virus neutralizing activity (Trible et al., 2012). It has been suggested that the level of neutralizing PCV2-specific antibodies is an indicator of protection against PCVDs (Fort et al., 2007; Trible et al., 2012).

In Switzerland, PCV2-SD was described first by Borel et al. in 2001. However, it was shown retrospectively that PCV2 was present in the Swiss pig population at least as early as 1979 (Wiederkehr et al., 2009). In a prospective study from 2001/02,

PMWS was not the main reason for wasting in weaned piglets (Staebler et al., 2004). From 72 wasting piglets out of 26 farms only 4 pigs (5%) showed histologically typical PMWS lesions and 11 showed moderate IHC positivity. However, 70% had PCV2-specific antibodies measured by IPMA (Staebler et al., 2004). In spite of the high sero-prevalence, PMWS was diagnosed more and more in Switzerland since 2003, first in regions of Switzerland with high pig density (Welti et al., 2012). The sudden onset of the epizooty was explained with a genetic shift from PCV2a to PCV2b-CH subtype (Wiederkehr et al., 2009). It should be mentioned, that then as now, Switzerland is free of PRRSV as well as enzootic pneumonia and actinobazillosis (Sidler et al., 2015). Therefore, a respiratory disease complex did not exist in Switzerland during the PCV2 epizooty. Since the introduction of piglet vaccination in the third week of life with a one-shot piglet vaccine in 2008, PMWS has been decreased dramatically. Today about 90% of the piglets are vaccinated and PMWS has nearly disappeared.

The aim of this study was to examine PCV2 specific antibody titre profils in the Swiss pig population in different regions with different size of pig farms at the pre-epizootic, epizootic and post-epizootic period.

2 Material and Methods

2.1 Materials

600 serum samples of slaughter pigs of the serum database of the Institute of Virus and Immunprophylaxis (IVI), Mittelhäusern, Switzerland, were analized, 200 samples of each time points of the years 1996/97, before the epizooty, during the epizooty in 2006, but before mass vaccination and 2011, two years after piglet mass vaccination. The serum samples were chosen randomly and according to the pig densitiy and farm size in the various regions in Switzerland. The regions are characterized as follow: Canton Berne (small farm size), Canton Lucerne (moderate farm size and highest pig density), eastern Switzerland (largest farm size) and the rest of Switzerland.

2.2 Methods

2.2.1 Serological examinations

A competitive ELISA (SERELISA® PCV2 Ab Mono Blocking Systems, Synbiotics Corporation Europe SAS, Lyon) was used for antibody (IgG) detection. The completion of the test, data analysis and transformation of the data into ELISA units (EU) were done according to the manufacturer's instructions and a published reference (Guillossou et al., 2008). We supplemented the assay with two additional controls to the sera dilutions suggested by the manufacturer. First, we used an additional positive and negative control serumg to check plate antigen coating homogeneity. Secondly, we normalized S-values among individual plates with the aid of a known serum (Kurmann et al., 2011). Immunoglobulin M (IgM) was measured using INGEZIM CIRCOVIRUS IgG/IgM (Ingenasa, Madrid), a capture immunoenzymatic assay specific IgM antibody detection to PCV2. For the IgM analysis, 50 samples of each period were randomly chosen and tested.

2.2.2 Statistics

Data editing was done using Excel (Microsoft Inc.) and the statistical analyses were done using Stata Software (StataCorp., 2011; Stata Statistical Software: Release 12; College Station, TX, USA: StataCorp LP). Analyses were carried out with ANOVA (Bonferroni corrected) and Chi-Square-Test. A p-value of ≤ 0.05 was considered as significant.

To compare the different titre heights we put two arbitrary thresholds at titre heights of 1000 EU and 8000 EU.

3 Results

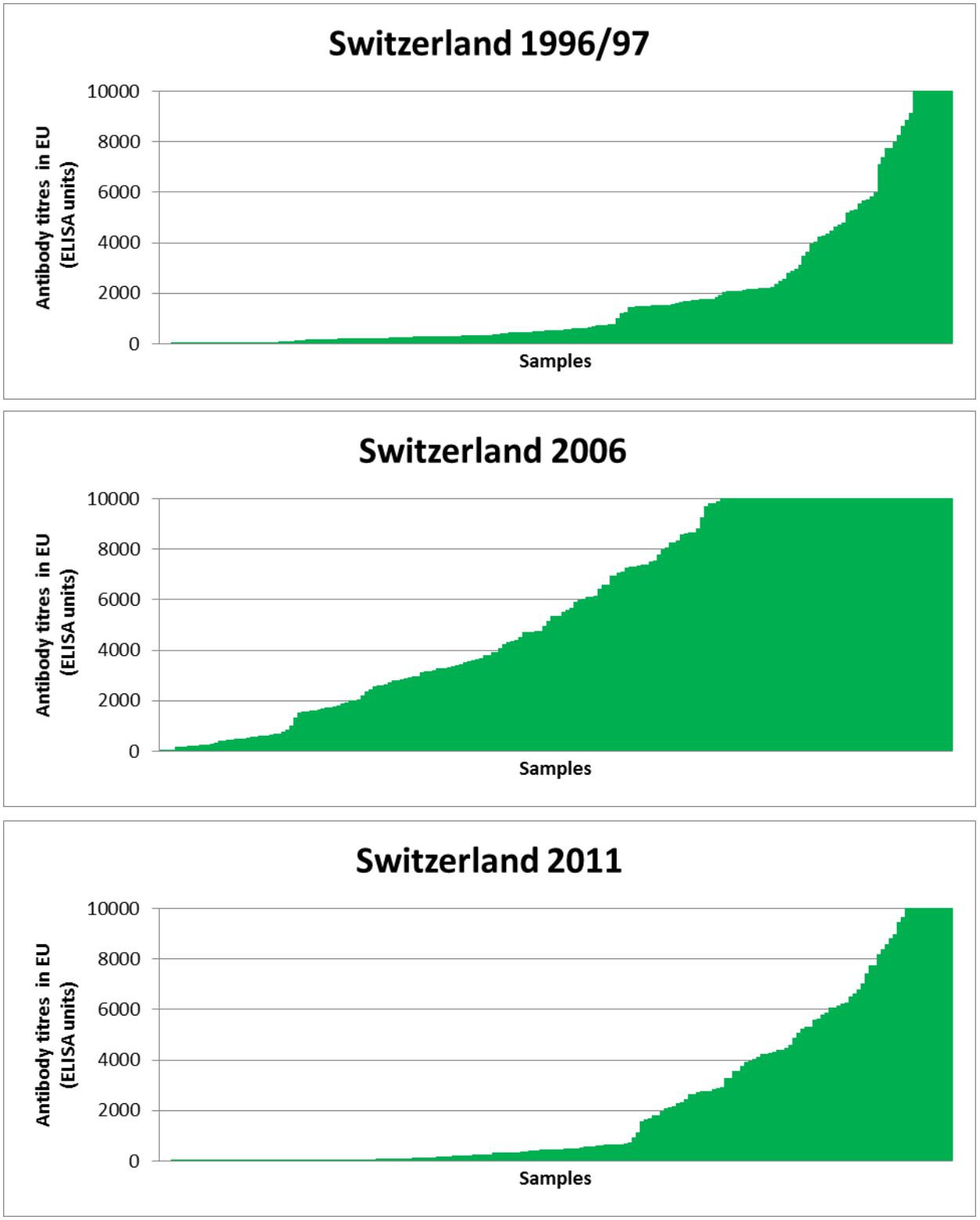
3.1 Distribution of PCV2 antibodies in Switzerland in 1996/97, 2006 and 2011

Development of the PCV2 specific antibody titres between the different time points is shown in figure 1. The titres varied heavily between the different periods.

In 1996/97, 43% of the samples had an antigen titre above 1000 EU. Only 7% of the samples reached titres >8000 EU. In 2006 the number of titres >1000 EU rose from 43% to 84% and 37% of the samples showed titres >8000 EU ($p<0.05$). After mass vaccination of the piglets in 2011, the numbers of IgG positive serum samples decreased significantly from 84% to 40% as well as the number of samples with titres >8000 EU from 37% to 10% ($p<0.05$). However there is no statistically significant difference between the data from 1996/97 and 2011 ($p=0.83$).

Two of the samples of 2006 were positive for anti-PCV2 IgM antibodies; all the other samples were negative.

Figure 1: Distribution of PCV2-specific antibodies in Switzerland in 1996/97, 2006 and 2011 of 200 fattening pigs per period.



3.2 Regional distribution of anti-PCV2 IgG antibodies in 1996/97, 2006 and 2011

The regional differences mirror the Swiss wide results and are shown in table 1 and figure 2 and 3. All the regions have statistically significant differences in titre height between 1996/97 and 2006 and also 2006 and 2011 ($p<0.05$). There are no statistically significant differences comparing 1996/97 and 2011. In the different years, there are no statistically significant differences comparing the various regions.

Table 1: Distribution of the antibody titres in the different regions of Switzerland in the three different years.

Region	n= samples	**1996/97** >1000 / >8000 EU (%)	**2006** >1000 / >8000 EU (%)	**2011** >1000 / >8000 EU (%)
Canton Lucerne	60	40 / 3	87 / 43	28 / 8
Canton Berne	35	54 / 9	86 / 46	44 / 9
Eastern Switzerland	52	42 / 4	79 / 27	45 / 8
Rest of Switzerland	53	40 / 13	83 / 28	42 / 12

Figure 2: ANOVA results of the titre comparison (the 4 regions of Switzerland, median and 95% confidence interval)

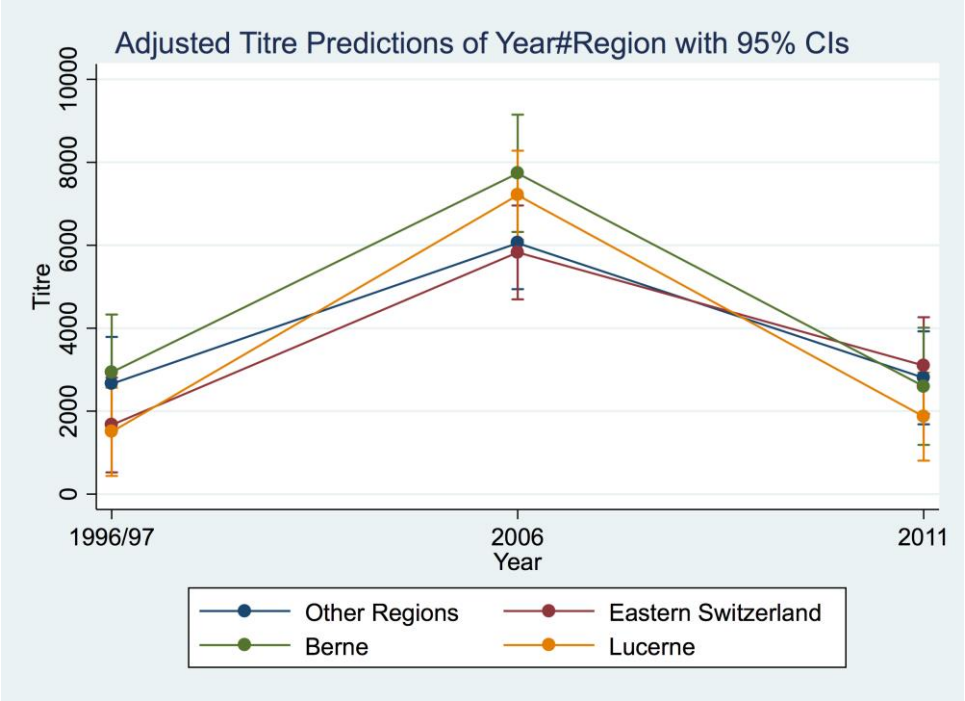
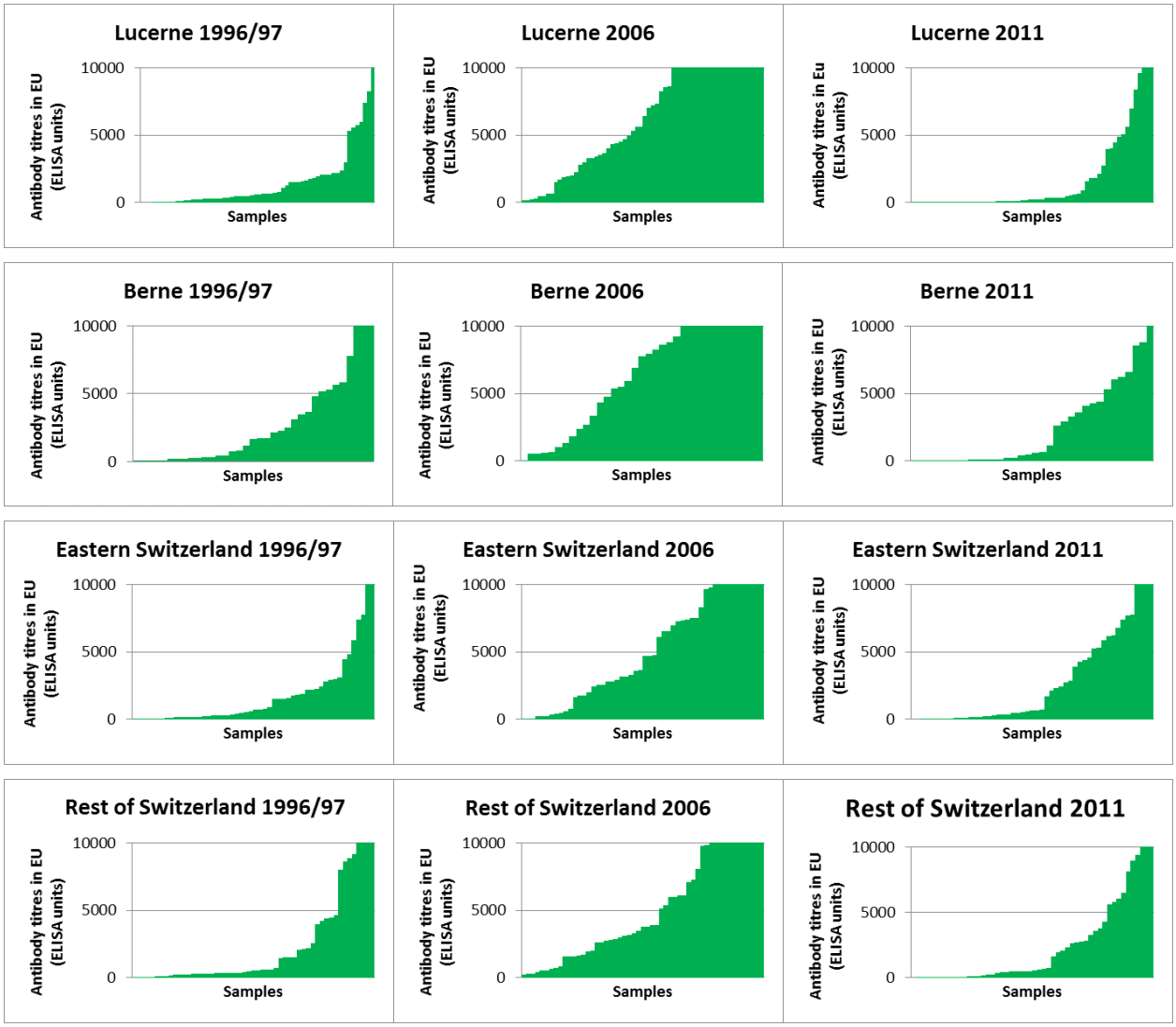


Figure 3: IgG Antibody titres from different regions in Switzerland at the preepizootic stage (1996/97), nearly at the zenith of the PMWS epizooty (2006) and two years after mass vaccination (2011).



4 Discussion

In the Swiss pig population, the number of PCV2-seropositive pigs as well as antibody titres increased significantly between 1996/1997 and 2006. This augmentation is due to an increased and prolonged virus excretion and extended viremia of sick pigs. As a result, infection pressure has risen with the accelerated number of diseased pigs during the epizooty. Two years after mass vaccination of piglets in the third week of life, the number of seropositive pigs and the titre returned to the pre-epizootic level. It can be speculated, that after piglet vaccination in the third week of life, PCV2-specific IgG decrease strongly till slaughtering and the vaccination is not protecting as long as the vaccine manufacturers described. However, own studies have indicated that after vaccination in the third week of life, the number of seroconverted pigs and the antibody titres of 30 kg pigs were not different from those of 105 kg body weight (data not shown). We also tested 50 randomly selected sera from each of the 3 time-intervals for PCV2-specific-IgM. Only two pigs of the year 2006 were IgM positive, suggesting that none of the vaccinated pigs of 2011 had a late PCV2-reinfection, despite low PCV2-specific-IgG-antibodies.

There was no statistical significant difference in the different regions of Switzerland regarding the number of positive and height of antibody titres.

Low antibody response as well as lack of antibody development does not correlate with lack of protection (Kekarainen et al., 2010). Fenaux et al. (2004) demonstrated that not all vaccinated piglets seroconverted after vaccination, but they still were protected in a challenge model. Several studies have shown that vaccination in the 3rd week of life induces both, humoral and cell-mediated immune response, which protects the piglets from disease. PCV2 specific interferon-γ-secreting cells were increased 3 weeks after vaccination. Interferon-γ-secreting cells play an important role in the viral clearance (Fort et al., 2009; Martelli et al., 2011). Before vaccination more than 70% of the piglets exhibited $>10^7$ DNA copies/ml blood, while in all vaccinated pigs the virus load was lower than 10^6 DNA copies (Martelli et al., 2011).

Normally, the first contact with a pathogen or the first application of the vaccine leads to a stimulation of memory cells and very few IgG-secreting plasma cells. However, a second contact or a second vaccination leads to a massive increase of IgG. The aim

of a one-shot-vaccination is to stimulate the immune system in all vaccinated animals. The booster, irrespectively, the stimulation of the IgG-secreting plasma cells has to be done by the ubiquitously occurring PCV2.

Inducing PCV2 neutralizing antibodies, vaccination leads to a significant but an incomplete virus clearance (Fort et al., 2008; Fort et al., 2009; Martelli 2011; Trible et al., 2011) and consequently to a reduction of infection pressure. In a six-year lasting vaccination program in the USA, the number of PCV2 viremic finisher pigs dropped from 82.6% in 2006 to 17.2% in 2012 and PCV2 negative finisher farms increased from 0.5 to 52.1% (Dvorak et al., 2016). There was a 22-fold decrease in median viral levels and the percentage of PCV2-specific antibody positive pigs showed a marked decrease from 78.8% in 2006 to 19% in 2006 (Puvanendiran et al., 2011). The return to the pre-epizootic level considering PCV2 antibody levels two years after mass vaccination can be explained by the hypothesis, that infection pressure is not strong enough to stimulate the IgG-producing plasma cells. As a result of long lasting mass vaccination, in Switzerland PCV2-SD has nearly disappeared, in accordance to Afgahah et al. (2016). In case of vaccination, virus shedding is decreased significantly and therefore it can be speculated that long lasting vaccination may lead to a PCV2-elimination over time.

In conclusion, a long lasting vaccination programme is able to reduce infection pressure prominently. With commercial ELISA methods, the total amount of PCV2 specific IgG can be measured and they cannot be distinguished from neutralizing antibodies. Nevertheless, these ELISA are suitable for surveillance of infection pressure in a herd or in a country.

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